

# Purinergic receptors in the splanchnic circulation

Manuela Morato · Teresa Sousa ·  
António Albino-Teixeira

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**Abstract** There is considerable evidence that purines are vasoactive molecules involved in the regulation of blood flow. Adenosine is a well known vasodilator that also acts as a modulator of the response to other vasoactive substances. Adenosine exerts its effects by interacting with adenosine receptors. These are metabotropic G-protein coupled receptors and include four subtypes, A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>. Adenosine triphosphate (ATP) is a co-transmitter in vascular neuroeffector junctions and is known to activate two distinct types of P2 receptors, P2X (ionotropic) and P2Y (metabotropic). ATP can exert either vasoconstrictive or vasorelaxant effects, depending on the P2 receptor subtype involved. Splanchnic vascular beds are of particular interest, as they receive a large fraction of the cardiac output. This review focus on purinergic receptors role in the splanchnic vasomotor control. Here, we give an overview on the distribution and diversity of effects of purinergic receptors in splanchnic vessels. Pre- and post-junctional receptormediated responses are summarized. Attention is also given to the interactions between purinergic receptors and other receptors in the splanchnic circulation.

**Keywords** Adenosine receptors · purinergic · P2X receptors · P2Y receptors · Splanchnic vasculature

## Introduction

Almost fifty years separate the first report of extracellular actions of purine nucleotides and nucleosides by Drury and Szent-Györgyi in 1929 [39] and the definition of purinergic receptors by Burnstock in 1976 [18]. In the last few decades, a lot of research has been made in what concerns purinergic receptors and it is nowadays established that extracellular nucleotides and nucleosides are released by cells to provide the primary components for purine responses [43]. Purinergic receptors are important regulators of the blood flow, since they mediate vasoconstriction, vasodilation and neurotransmitter release. The splanchnic circulation is composed of gastric, small intestinal, colonic, pancreatic, hepatic and splenic circulations, arranged in parallel with one another. The major arteries supplying the splanchnic organs are the celiac artery, the superior mesenteric artery and inferior mesenteric artery, which branch off from the aorta and give rise to smaller arteries that anastomose extensively. Splanchnic blood is drained to the liver via the portal vein, and flows from the liver to the inferior vena cava via the hepatic veins [101, 127]. Since the splanchnic circulation receives about 25% of the cardiac output, alterations in the splanchnic circulation may have important clinical consequences. This review will focuss on the distribution and function of purinergic receptors particularly in vessels of the splanchnic circulation.

According to the IUPHAR Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) [51], purinergic receptors comprise adenosine receptors and P2 receptors, given that their endogenous agonist is

M. Morato · T. Sousa · A. Albino-Teixeira  
Institute of Pharmacology and Therapeutics,  
Faculty of Medicine and IBMC, University of Porto,  
Porto, Portugal

M. Morato · T. Sousa  
Pharmacology Department, Faculty of Pharmacy,  
University of Porto,  
Porto, Portugal

A. Albino-Teixeira (✉)  
Faculty of Medicine of Porto,  
Institute of Pharmacology and Therapeutics,  
Alameda Prof. Hernâni Monteiro,  
4200–319 Porto, Portugal  
e-mail: [albinote@med.up.pt](mailto:albinote@med.up.pt)

adenosine or adenosine triphosphate (ATP), respectively. P2 receptors are further divided in P2X receptors, which are ionotropic ligand-gated ion channel receptors, and P2Y receptors, which are metabotropic G protein-coupled receptors.

There are four different adenosine receptors, denoted A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub> [52]. All of them have been cloned in several different animal species, including human. Adenosine receptors are G protein-coupled receptors. Classically, they have been distinguished by those that couple to G<sub>i</sub> proteins, the adenosine A<sub>1</sub> and A<sub>3</sub> receptors, and those that are coupled to G<sub>s</sub> proteins, the adenosine A<sub>2A</sub> and A<sub>2B</sub> receptors. Nowadays, adenosine receptors are viewed as pleiotropic receptors since for the four of them there is evidence of distinct profiles of coupling to different G proteins and to different transducing systems, according to their degree of activation and with their particular localization [52].

P2X receptors are a family of seven proteins (P2X<sub>1</sub>–P2X<sub>7</sub>) [77]. All P2X receptors are ligand-gated ion channel-type, with almost equal permeability to Na<sup>+</sup> and K<sup>+</sup>, and significant permeability to Ca<sup>2+</sup> [46]. The seven P2X subunits form functional receptors that are named homomeric P2X receptors when expressed alone or heteromeric P2X receptors if they result from the combination of different subunits. Often native cell types express multiple P2X subunit mRNA transcripts and multiple P2X subunits. It is not clear how many subunits are required in each heteromer, which ones dominate, or if a greater number of heteromers can be formed [78, 121, 163]. P2X<sub>6</sub> subunits form only heteromeric channels and P2X<sub>7</sub> subunits only homomeric channels [173]. P2X receptors form as multimeric assemblies of channel subunits. The exact stoichiometry of the receptor remains unclear although studies have suggested that the channel may be comprised of at least three subunits [163].

The family of the P2Y receptors includes, for now, 14 different receptors. The P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub>, P2Y<sub>11</sub>, P2Y<sub>12</sub> and P2Y<sub>13</sub> receptors are the mammalian receptors, while the P2Y<sub>3</sub>, P2Y<sub>5</sub>, P2Y<sub>7</sub>, P2Y<sub>8</sub>, P2Y<sub>9</sub> and P2Y<sub>10</sub> receptors represent receptors cloned from non-mammalian vertebrates or receptors that are currently under functional characterization [1]. Based on (1) sequence homology, (2) the presence of key amino acids essential for receptor activation, (3) chromosomal colocalization, (4) similarity of the cognate ligand, and (5) identification of uridine diphosphate (UDP)-glucose in the extracellular space, the IUPHAR Subcommittee recently renamed the UDP-glucose receptor the P2Y<sub>14</sub> receptor [1].

P2Y receptors are metabotropic G protein-coupled receptors and although P2Y receptors couple to specific G proteins, one given P2Y receptor can couple to functionally distinct G proteins and signalling pathways [2]. This

provides the opportunity for agonist-specific signaling involving distinct active conformations of the receptor which raises the possibility of pursuing selective targets with therapeutic relevance. However, there are no studies on this issue in what concerns the splanchnic circulation. Pharmacologically, P2Y receptors can be subdivided into the adenine-nucleotide-preferring receptors mainly responding to adenosine diphosphate (ADP) and ATP (human and rodent P2Y<sub>1</sub>, P2Y<sub>12</sub> and P2Y<sub>13</sub>, and human P2Y<sub>11</sub>), the uracil-nucleotide-preferring receptors (human P2Y<sub>4</sub> and P2Y<sub>6</sub>) responding to either uridine triphosphate (UTP) or UDP, receptors of mixed selectivity (human and rodent P2Y<sub>2</sub> and rodent P2Y<sub>4</sub>), and receptors responding only to UDP-glucose and UDP-galactose (P2Y<sub>14</sub>) [2].

### Adenosine receptors in the splanchnic circulation

Despite the existence of several studies describing a role for adenosine in the regulation of splanchnic circulation [48, 55, 87, 118, 150, 151], there is little information concerning to adenosine receptors distribution in these vascular beds. Till now, expression of adenosine receptors in splanchnic vessels has been evaluated only in the mesenteric vasculature [36, 71]. Western-blot analysis and quantitative densitometry revealed that mesenteric microvessels from Wistar rats express all the adenosine receptors subtypes, i.e., A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> receptors. A<sub>2A</sub> and A<sub>3</sub> receptors were found to be abundantly expressed in these vessels [71]. Similar results were also found by reverse transcriptase polymerase chain reaction (RT-PCR) studies in the Sprague Dawley rat mesenteric vascular bed, thus suggesting an important physiological role for A<sub>2A</sub> and A<sub>3</sub> receptors in the regulation of intestinal blood flow [36]. Binding assays with selective ligands for A<sub>2A</sub> receptors also evidenced the presence of this receptor subtype in the rabbit mesenteric artery [31]. There are, however, some differences concerning to adenosine A<sub>1</sub> receptor expression. A<sub>1</sub> receptor protein appears to be significantly expressed in mesenteric microvessels from Wistar rats [71], while A<sub>1</sub> receptor mRNA was undetected in the mesenteric vascular bed from Sprague Dawley rats [36]. These contrasting results may be due to technique limitations or to differences between rat strains. It is also possible that the authors studied different segments of the mesenteric vascular bed. While Jackson et al [71] specified that 2nd and 3rd order branches of the mesenteric vasculature were used, Donoso et al [36] just refers to the mesenteric arterial bed. Moreover, the inclusion of sympathetic nerve fibers in the homogenate could also account for the discrepancy in the A<sub>1</sub> receptor expression.

Table 1 summarizes the information on the distribution of adenosine receptors in vessels of the splanchnic circulation.

**Table 1** Distribution of adenosine receptors in vessels of the splanchnic circulation

Adenosine Receptor	Preparation	Technique	Expression	Reference
<b>A<sub>1</sub></b>	Rat mesenteric microvessels	Western Blot and quantitative densitometry	Strongly expressed	[71]
	Rat mesenteric vascular bed	RT-PCR	Not detected	[36]
<b>A<sub>2A</sub></b>	Rat mesenteric microvessels	Western Blot and quantitative densitometry	Very strongly expressed	[36, 71]
	Rat mesenteric vascular bed	RT-PCR	Strongly expressed	[31]
	Rabbit mesenteric artery	Binding assay		
<b>A<sub>2B</sub></b>	Rat mesenteric microvessels	Western Blot and quantitative densitometry	Low density	[71]
	Rat mesenteric vascular bed	RT-PCR	Weakly expressed	[36]
<b>A<sub>3</sub></b>	Rat mesenteric microvessels	Western Blot and quantitative densitometry	Very strongly expressed	[71]
	Rat mesenteric vascular bed	RT-PCR	Strongly expressed	[36]

### Effects mediated by adenosine receptors in the splanchnic circulation

Considerable evidence indicates that adenosine acts as a modulator of sympathetic neurotransmission, exerting either inhibitory or facilitatory effects. The regulation of noradrenaline release by adenosine has been observed in several vascular (tail artery, saphenous vein, portal vein, mesenteric artery, hepatic artery, pancreatic-duodenal arteries) [14, 33, 68, 69, 176] and non-vascular tissues (kidney, adrenal medulla, vas deferens, heart) [42, 84, 132, 174, 186].

Adenosine inhibitory effect on noradrenergic transmission has been generally attributed to the activation of prejunctional A<sub>1</sub> receptors [17, 34, 58, 74, 92, 158]. With respect to the splanchnic circulation, the majority of studies also points to a sympathoinhibitory role of adenosine mediated via A<sub>1</sub> receptors. In rabbit mesenteric arteries, adenosine and its analogs depressed neuroeffector transmission [68]. The rank order of potency of various agonists, R-N<sup>6</sup>-(phenylisopropyl-adenosine) (R-PIA)  $\cong$  5'-(N-ethyl-carboxamido)-adenosine (NECA) > S-N<sup>6</sup>-(phenylisopropyl-adenosine) (S-PIA) > adenosine, suggested that these actions were mediated by A<sub>1</sub> receptors [68].

An A<sub>1</sub> receptor with a modulatory effect on sympathetic neurotransmission was also identified in other vessels and species. In the rabbit portal vein, adenosine also inhibited the contractile response evoked by adrenergic nerve stimulation via activation of presynaptic adenosine receptors of the A<sub>1</sub> subtype [14]. In the hamster mesenteric arterial bed, sympathoinhibition was also shown to be mediated by prejunctional A<sub>1</sub> receptors [136]. A similar role was also observed for this adenosine receptor subtype in the mesenteric artery from Wistar rat. Adenosine, chloroadenosine (CADO) and NECA inhibited noradrenaline release while the selective A<sub>1</sub> receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) significantly reduced the inhibition induced by adenosine or its analogs [153].

In contrast with the studies describing the involvement of prejunctional A<sub>1</sub> receptors in sympathoinhibition, Donoso et al. recently observed that A<sub>2A</sub> and A<sub>3</sub> receptors

reduced the noradrenaline overflow from the nerve terminals of the Sprague Dawley mesenteric arterial bed [36]. The additive inhibitory effects of selective A<sub>2A</sub> and A<sub>3</sub> agonists and the significant increase in noradrenaline release elicited by the simultaneous blockade of A<sub>2A</sub> and A<sub>3</sub> receptors, suggest that these receptors are also able to decrease sympathetic transmission. Furthermore, these receptors are markedly expressed in the rat mesenteric vascular bed [36]. In the rat portal vein, it was also described that the sympathoinhibitory effect of adenosine and its analogs is mediated by prejunctional adenosine receptors with characteristics of the A<sub>2</sub> subtype [75].

Despite ample evidence describing the adenosine ability to inhibit noradrenergic transmission, there is a lack of experimental data supporting a physiological role for endogenous adenosine as a modulator of sympathetic neurotransmission. In a rat model of prolonged sympathetic nerve stimulation associated with decreased tissue perfusion, the administration of 1,3-dipropyl-8-sulphophenylxanthine (DPSPX), a non selective antagonist of A<sub>1</sub>/A<sub>2</sub> receptors, did not affect the mesenteric blood flow nor the noradrenaline spillover [83]. DPSPX also failed to attenuate noradrenergic neurotransmission during hemorrhagic hypotension [70]. Similar results were also observed in the hamster mesenteric arterial bed, where the application of DPCPX had no effect on contractile responses elicited by electrical field stimulation [136]. It has been described that activation of presynaptic  $\alpha_2$ -adrenoceptors reduces the inhibitory effect on noradrenaline release of A<sub>1</sub>-receptor activation [4, 15, 91]. It is possible that, in splanchnic vascular beds, the adenosine inhibitory effect on transmitter release is revealed when presynaptic  $\alpha_2$ -adrenoceptors are blocked, as previously observed in other tissues [58, 61, 84]. These results suggest that endogenous adenosine may not reach a sufficient level to exert a physiological effect within the neuroeffector junction. However, it remains possible, that under pathophysiological conditions or in tissues with higher metabolic demand, endogenous adenosine may assume a significant role as a modulator of sympathetic neurotransmission.

It is a well known fact that sympathetic neurons release both noradrenaline and ATP, which function as co-transmitters [156, 157]. Examples of this adrenergic-purineric cotransmission have also been observed in splanchnic vascular tissues such as mesenteric artery [181], mesenteric vein [155] and hepatic artery [12]. Activation of adenosine A<sub>1</sub> receptors by exogenous applied adenosine or its analogues has been shown to inhibit the release of both adrenergic and purineric transmitters [38]. Moreover, the A<sub>1</sub>-receptor mediated inhibition of ATP release appears to be more pronounced than the inhibition of noradrenaline overflow [38]. Regarding the splanchnic circulation, in rabbit mesenteric arteries, the activation of presynaptic A<sub>1</sub> receptors by adenosine also seems to inhibit ATP release [68]. Furthermore, in canine splenic arteries, the continuous infusion of ATP inhibited both noradrenaline and ATP releases [187]. This inhibitory effect was attributed to the activation of A<sub>1</sub> receptors by adenosine readily generated from ATP and was partially reversed by the A<sub>1</sub>-adenosine receptor antagonist, 8-phenyltheophylline [187].

In addition to its inhibitory action on adrenergic and purineric neurotransmission, A<sub>1</sub> receptors also appear to regulate non adrenergic non cholinergic transmission (NANC). The release of the NANC transmitter, calcitonin gene-related peptide, during perivascular nerve stimulation in rat mesenteric arterial bed was inhibited in the presence of adenosine receptor agonists and this effect was shown to be mediated by prejunctional A<sub>1</sub> receptors [146]. This interpretation was based in the order of potency obtained for the adenosine receptor agonists tested, N<sup>6</sup>-ciclopentyladenosine (CPA) ≥ R-PIA ≥ NECA ≥ CADO ≥ S-PIA and in the effects obtained in the presence of selective A<sub>1</sub> or A<sub>2</sub> receptors antagonists (DPCPX and 3-[4-[2-[[6-amino-9[(2R,3R,4S,5S)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]purin-2-yl]amino]ethyl] phenyl]propanoic acid (CGS21680), respectively) [146].

Adenosine elicits vasodilatation in several splanchnic vessels, leading to an increase in blood flow and a decrease in vascular resistance. The vasorelaxant response to adenosine or its analogs has been observed in mesenteric, hepatic, gastric and pancreatic vascular beds [23, 24, 87, 102, 159, 194]. The spleen circulation appears to be an exception, since the splenic arterial conductance was shown to be reduced in response to the administration of the adenosine analog, CGS21680 [120]. There is consensus that the vasodilatory effects of adenosine are mediated by A<sub>2</sub> receptors. However, it is still a matter of debate which A<sub>2</sub> receptor subclass, i.e., A<sub>2A</sub> or A<sub>2B</sub>, is involved in these vasorelaxant responses. In mesenteric vessels both A<sub>2A</sub> and A<sub>2B</sub> receptors seem to contribute to vasodilatation. Indeed, it was previously observed that these A<sub>2</sub> receptor subtypes are expressed in the mesenteric vascular bed, although A<sub>2B</sub> receptors are weakly expressed in comparison to A<sub>2A</sub>

receptors [71]. A<sub>2A</sub> receptor-mediated vasodilatory effects have been observed in the rat and rabbit mesenteric artery [31, 64, 79, 133]. These effects appear to involve the opening of potassium channels subsequent to A<sub>2A</sub> receptor activation. As previously referred, A<sub>2</sub> receptors are coupled to G<sub>s</sub> proteins, which activate adenylyl cyclase leading to an increase in intracellular cAMP. The elevation of cAMP further activates protein kinase A, which opens potassium channels probably through a phosphorylation step [79].

A vasodilatory response to adenosine mediated by A<sub>2A</sub> receptors was also observed in the portal vein and in the pancreatic vascular bed [59, 87].

In contrast, other studies in the rat mesenteric artery point to an A<sub>2B</sub>-mediated vasodilatation, based on the potency order for NECA, adenosine and CGS21680 (NECA > adenosine > CGS21680). In these works, the selective A<sub>2A</sub> receptor antagonist failed to induce vasodilatation, unless high concentrations were used [131, 145]. A<sub>2B</sub> receptors seem to be primarily located on the smooth muscle. However, their contribution to vasodilatation appears to occur only in conditions of blockade of adenosine uptake. When the uptake is operational, adenosine and its analogs seem to activate a xanthine-resistant site which may be intracellular [131].

Adenosine-induced vasodilatation in splanchnic vessels appears to be dependent, at least in part, on the presence of a functional endothelium [167]. However, it does not seem to involve the release of nitric oxide (NO), since the vasodilatory response to adenosine or its analogs was not reduced by the blockade of NO synthesis in the isolated mesenteric artery and gastric resistance vessels [25, 64, 145].

Although it is well established that adenosine-induced vasodilation is mediated by A<sub>2</sub> receptors, the administration of the selective A<sub>1</sub> antagonist, DPCPX, was shown to abolish the glucose-induced increase in pancreatic islet blood flow. This surprising finding suggests that A<sub>1</sub> receptors may also contribute to the vasorelaxant response in this vascular bed [22].

The magnitude of adenosine vasodilatory effects seems to vary with the caliber of the vessels. For instance, in the mesenteric arterial bed the pre-capillary arterioles showed the largest increases in diameter in response to adenosine, when compared to the effects in principal arterioles [103]. A similar effect was also observed in the venous side of the mesenteric circulation [103]. An opposite situation appears to occur in the hepatic vascular bed, where adenosine was shown to dilate the hepatic artery and the portal vein, but not the intrahepatic portal resistance vessels [23, 88].

Table 2 summarizes the information on the potencies of agonists and antagonists at adenosine receptors in the splanchnic circulation.

Just recently, the possibility of crosstalk between prejunctional facilitatory angiotensin II receptors and

**Table 2** Potencies of agonists and antagonists at adenosine receptors in the splanchnic circulation

Agonist/Antagonist	EC <sub>50</sub> (μM)	EC <sub>40</sub> (μM)	EC <sub>30</sub> (μM)	pEC <sub>50</sub>	pEC <sub>40</sub>	pEC <sub>20</sub>	pA <sub>2</sub>	Reference
<i>Adenosine receptors</i>								
8-SPT							5.63±0.26	[131]
<i>Adenosine A<sub>1</sub> receptor</i>								
Adenosine		4.7				5.8±0.1		[68]
	14.9±4.0							[136]
NECA		0.028				7.6±0.2		[59]
	18.2±0.7							[68]
			0.15 (0.02–0.42)					[136]
CADO	0.0267±0.001							[146]
			0.46 (0.18–0.87)					[153]
CPA	0.00108±0.0009							[146]
						7.5±0.1		[136]
R-PIA	0.00186±0.0005							[68]
		0.018						[146]
S-PIA	0.0366±0.0019							[68]
		0.83						[146]
<i>Adenosine A<sub>2A</sub> receptor</i>								
NECA	0.06±0.02							[31]
CGS21680	0.02±0.01							[31]
CADO	0.64±0.11							[31]
ZM241385							7.65±0.25	[131]
<i>Adenosine A<sub>2B</sub> receptor</i>								
Adenosine					4.58±0.08			[131]
								[111]
NECA				6.16±0.11				[131]
CPA					4.54±0.12			[131]
R-PIA				5.31±0.13				[131]
IB-MECA				5.38±0.06				[131]
<i>Adenosine A<sub>2A</sub>/A<sub>3</sub> receptor</i>								
Adenosine	0.2012±0.0342							[36]
NECA	0.0026±0.0009							[36]
CGS21680	0.0035±0.0015							[36]
CPA	0.380±0.246							[36]
IB-MECA	0.0033±0.00085							[36]

Predominant receptor considered to be involved on the basis of functional data and receptor localization studies. Values ± S.E.M. or 95% confidence interval (in parenthesis). EC<sub>50</sub>, EC<sub>40</sub> and EC<sub>20</sub> represent the molar concentration of the agonist needed to cause 50%, 40% and 20% of the maximum response, respectively; pEC<sub>50</sub>, pEC<sub>40</sub> and pEC<sub>20</sub> are the negative logarithme to base 10 of the EC<sub>50</sub>, EC<sub>40</sub> and EC<sub>20</sub> of an agonist, respectively; pA<sub>2</sub> is the negative logarithme to base 10 of the molar concentration of the antagonist that makes it necessary to double the concentration of the agonist needed to elicit the original response. 8-sulphophenyltheophylline (8-SPT); N<sup>6</sup>-(3-iodo-benzyl)-adenosine-5'-N-methyluronamide (IB-MECA); 5'-(N-ethylcarboxamido)-adenosine (NECA); chloroadenosine (CADO); N<sup>6</sup>-ciclopentyladenosine (CPA); R-N<sup>6</sup>-(phenylisopropyl)-adenosine (R-PIA); S-N<sup>6</sup>-(phenylisopropyl)-adenosine (S-PIA); 3-[4-[2-[[6-amino-9[(2R,3R,4S,5S)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]purin-2-yl]amino]ethyl]phenyl]propanoic acid (CGS21680); [<sup>3</sup>H]-4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM241385).

inhibitory adenosine and P2 receptors has emerged. It was described that in the rat mesenteric vein, but not in the artery, tonic activation of adenosine A<sub>1</sub> receptors, but not P2X receptors, amplify the facilitatory effect of angiotensin II on noradrenaline release induced by electrical field stimulation [169]. Adenosine-angiotensin II interactions were also described in the mesenteric vasculature of rats chronically infused with DPSPX, a non selective antagonist

of A<sub>1</sub>/A<sub>2</sub> adenosine receptors [106]. These rats have been shown to develop hypertension, marked vascular hypertrophy and hyperplasia, activation of the renin-angiotensin system and altered vascular reactivity [3, 106, 107, 126, 160]. Recently, it was described that the angiotensin II facilitatory effect on noradrenaline release was increased in mesenteric artery from DPSPX-treated rats. In contrast, the acute exposure of mesenteric vessels from control rats to



DPSPX *in vitro* did not affect the facilitatory action of angiotensin II [106]. Also, in rat perfused mesentery intra-arterial infusions of DPSPX did not alter the effects of intra-arterial or intravenous angiotensin II on periaarterial nerve stimulation or on vascular tone [66]. It is possible, however, that acute *versus* chronic blockade of adenosine receptors has different consequences and that the putative crosstalk is only evident after prolonged exposure to DPSPX. Further studies are needed to better characterize these interactions.

Portal hypertension results in both increased portal pressure and reduced splanchnic vascular resistance, leading to marked splanchnic hyperemia [141]. The pronounced arterial vasodilation and increased systemic and mesenteric artery blood flow observed in portal hypertension leads to systemic hypotension, despite an increased blood volume and cardiac output. The adenosine-mediated mesenteric vasodilation depends on a functional endothelium [32, 64, 167]. Adenosine vasodilation is commonly described to involve A<sub>2A</sub> receptor activation, although A<sub>2B</sub> receptors may also play a role [6, 49, 124]. There is evidence of both A<sub>2A</sub> [64] or A<sub>2B</sub> receptor-mediated vasodilation [131, 145] in the mesenteric artery. Experimental differences (species, methodology) may explain the discordant information regarding to the adenosine receptor responsible for the relaxant response in mesenteric arteries of the rat [31]. An A<sub>2A</sub> receptor-mediated relaxation of mesenteric arteries can be observed in the rat, as described in the rabbit [31, 64]. The importance of this adenosine-induced vasodilation is further stressed by the fact that chronic blockade of adenosine receptors leads to hypertension [3].

The vascular responses to vasoactive agents are modified in portal hypertension [28, 53, 89]. The rabbit model of partial portal vein ligation to study portal hypertension is characterized by the development of portal-systemic shunting [5, 26]. In rabbits with portal hypertension the vasodilation of the cranial mesenteric artery induced by adenosine is decreased both *in vitro* [30] and *in vivo* [100]. In portal hypertensive animals, there was mainly a decreased potency but also a decreased efficacy of all tested adenosine agonists compared to normal animals. Concomitantly, there was a decreased adenosine plasma level and a decreased binding density of [<sup>3</sup>H]-CGS21680 and [<sup>3</sup>H]-4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM241385) to mesenteric artery membranes from portal hypertensive compared to normal rabbits [31]. The adenosine receptor responsible for the adenosine-induced relaxation of mesenteric arteries is of the A<sub>2A</sub> subtype and the reduced density of these A<sub>2A</sub> receptors may contribute for the reduced relaxant response to adenosine in the mesenteric arteries of portal hypertensive animals. Thus, de Brito et al. [31] hypothesized that hypertension-induced changes in the uptake and/or metabolism of plasma adenosine may have contributed for the

significant decrease in adenosine plasma levels observed in their study. The most likely explanation for the observed decrease in the potency and efficacy of adenosine receptor agonists to induce mesenteric relaxation in portal hypertensive rabbits resides on the observed decreased density of adenosine A<sub>2A</sub> receptors.

### P2X and P2Y receptors in the splanchnic circulation

In 1993, Bo and Burnstock [7] provided direct evidence for the existence of P2X receptors in the vascular smooth muscle using a radioligand binding assay and autoradiography with [<sup>3</sup>H]α,β-methylene ATP ([<sup>3</sup>H]α,β-meATP). Already in this study, the authors reported regional and species differences in the distribution of P2X receptors [7]: (1) medium- and small-sized arteries had higher densities than the elastic and large muscular arteries, and (2) in some large muscular arteries, such as the rabbit hepatic and mesenteric arteries, the outer region of the vessel had a higher density of receptors than the inner region. In the guinea-pig and rat portal veins, the longitudinal muscle has much higher densities of P2X receptors than does the circular muscle, while in the rabbit portal vein the circular muscle has higher density of P2X receptors than the longitudinal muscle [7]. In the rabbit mesenteric vein, very low quantities of [<sup>3</sup>H]α,β-meATP were observed [7]. [<sup>3</sup>H]α,β-meATP is almost ineffective as an agonist at some P2X receptors [165] and has a complex binding profile [104], which limits the interest of ligand-binding autoradiography using this ligand. Nevertheless, the results of Bo and Burnstock were further stressed and completed using different techniques, for the most part in the mesenteric vasculature. The presence of P2X<sub>1</sub>-immunoreactivity was observed in the rat mesenteric artery [54, 60, 62, 90], but also in arterioles of the submucous plexus of the guinea-pig ileum [184] and in rat and mouse pancreatic vascular smooth muscle [29]. Conflicting results have been obtained in what concerns the venous part of the circulation. Hansen and co-workers [60] observed P2X<sub>1</sub>-immunoreactivity in both the rat mesenteric artery and vein while Galligan and co-workers [54] described the presence of P2X<sub>1</sub>-immunoreactivity in rat mesenteric arteries, but not in mesenteric veins. Using RT-PCR, mRNA of P2X<sub>1</sub> receptors has been detected in rat hepatic [130] and mesenteric [129] arteries. However, there is a report of no detection of mRNA transcripts of P2X<sub>1</sub> receptors in the mesenteric artery of the rat [122]. Differences in the mesenteric arteries analysed can account for the conflicting results, since the former study used the superior mesenteric artery [122] while the latter study studied segments of superior and 2<sup>nd</sup>-order mesenteric arteries [129]. However, immunohistochemical analysis [57] revealed no major differences in the pattern of

P2X receptor isoform expression between the three sizes of vessel and may suggest that a novel additional subunit is expressed in large arteries.

Another pertinent question is where in the vascular wall are P2X<sub>1</sub> receptors located? In this way, the majority if not almost all, of the studies in which this issue was persecuted, have found P2X<sub>1</sub> receptors in the smooth muscle cell of the media [7, 29, 60, 90, 184]. Very recently, a study of Harrington and Mitchell [62] reported for the first time the presence of P2X<sub>1</sub>-immunoreactivity in all the three layers of the rat mesenteric artery: not only in the smooth muscle cells of the media, but also in the adventia and in the endothelium. Curiously, the small P2X<sub>1</sub>-receptor cluster identified by Hansen and co-workers was restricted to the adventitial surface of the media and to endothelial cells [60]. Opposedly to these studies, Lewis and Evans failed to detect P2X<sub>1</sub>-immunoreactivity on the endothelium [90]. It seems that P2X<sub>1</sub> receptors are not located on nerves since they are still identified after destruction of the sympathetic nerves with 6-hydroxydopamine [60].

Some controversy exists concerning the distribution of P2X<sub>2</sub> receptors in the splanchnic circulation. RT-PCR studies showed that P2X<sub>2</sub> receptors are either absent [122] or weakly expressed in rat mesenteric artery and rat hepatic artery [130]. In contrast, immunohistochemistry studies identify the presence of P2X<sub>2</sub> receptors in the nerves and endothelial cells of the rat mesenteric artery and vein [60], in nerve fibers of the perivascular plexuses of the guinea-pig ileum [184] and also in the smooth muscle of the rat mesenteric artery and vein [60] and pancreatic vascular bed [29]. Interestingly, two splice variants of the P2X<sub>2</sub> receptors have been identified in rat coronary artery and aorta [122].

Regarding the other P2X receptors, the P2X<sub>3</sub> receptor is weakly expressed both in the rat hepatic [130] and mesenteric [129] arteries. In contrast, the P2X<sub>4</sub> and the P2X<sub>7</sub> receptors are reported to be very strongly expressed in the rat hepatic [130] and mesenteric [129] arteries, although some authors described no detection of P2X<sub>4</sub> mRNA transcripts [122]. The P2X<sub>5</sub> receptor is strongly expressed in the rat hepatic artery [130] but weakly expressed in rat mesenteric artery [129]. Till now, the P2X<sub>6</sub> receptor was never detected in the splanchnic vasculature [129, 130].

In summary, with the exception of the P2X<sub>6</sub> receptor, all the other P2X receptors are expressed in the splanchnic circulation, at least in arteries. P2X<sub>1</sub>, P2X<sub>4</sub>, P2X<sub>5</sub> and P2X<sub>7</sub> receptors are strongly expressed while P2X<sub>2</sub> and P2X<sub>3</sub> receptors are weakly expressed. This difference may be associated with the particular role that each P2X receptor plays in the splanchnic circulation. However, functional studies only evidence the major role of P2X<sub>1</sub> receptor in mediating arterial vasoconstriction (see below). Even the late slow and sustained relaxation observed in response to ATP has been suggested to be mediated by P2X<sub>1</sub> receptors

(see below). Further studies are required to conclude about this issue since although it is now possible to block gene expression and raise mice with deletion of P2 receptors, the study of the P2X receptors is still held back by the lack of potent and selective antagonists.

Not so much information exists concerning the distribution of P2Y receptors in splanchnic vessels. In general, P2Y receptors are found both on vascular endothelial and smooth muscle cells [40, 138]. RT-PCR studies found mRNA fragments for P2Y<sub>1</sub>, P2Y<sub>2</sub> and P2Y<sub>6</sub> receptors in the rat [21] and mouse [177] mesenteric arteries, while P2Y<sub>4</sub> mRNA wasn't detected [21]. Moreover, P2Y<sub>1</sub>- and P2Y<sub>2</sub>-immunoreactivity was present in the smooth muscle of the rat pancreatic vascular bed [29]. Functional studies identify the presence of P2Y<sub>1</sub> receptors in the rat mesenteric vein [54] and P2Y<sub>2</sub> receptors in the guinea pig mesenteric vein [116].

Table 3 summarizes the information on the distribution of P2-purinoceptors in vessels of the splanchnic circulation.

### Effects mediated by P2X and P2Y receptors in the splanchnic circulation

The previous notion that noradrenaline was the single sympathetic neurotransmitter in vascular neuroeffector junctions has given place to the actual fact that ATP is a co-transmitter at this synapses [180]. In what concerns the splanchnic circulation, the first description of such co-release was reported by Su [164] in the rabbit portal vein. Since then, growing evidence emerged that ATP is a co-transmitter in the splanchnic circulation of the dog [9, 94, 113, 114, 144, 170], rabbit [140, 181], guinea-pig [8], and rat [19, 37, 44].

The co-release of noradrenaline and ATP from sympathetic nerve terminals may be subject to separate modulatory mechanisms. Differential autoinhibition has been suggested in the rat mesenteric artery [41]. In the rabbit ileocolic artery, activation of presynaptic  $\alpha_2$ -adrenoceptors preferentially inhibits the release of noradrenaline [16]. In canine splenic arteries, adrenergic neuron blockade with guanethidine [189] or inhibition of sodium channels by tetrodotoxin [188] mainly depresses the adrenergic rather than the purinergic component. Opposing to this, the purinergic component is depressed, rather than the adrenergic component, after irreversible degeneration of adrenergic nerve fibres due to cold storage [190] or with dipyrindamole [119]. Electrochemical and electrophysiological studies have also tried to clarify whether noradrenaline and ATP are co-stored and/or co-released. In vascular tissues not belonging to the splanchnic circulation, it seems that noradrenaline and ATP are released in parallel [110, 111], although these results are still controversial [109]. This

**Table 3** Distribution of P2-purinoceptors in vessels of the splanchnic circulation.

P2 Receptor	Preparation	Technique	Expression	Reference
<b>P2X<sub>1</sub></b>	Rat mesenteric artery	Immunohistochemistry	Yes	[60]
		Immunohistochemistry	Yes	[90]
		Immunohistochemistry	Yes	[54]
		Immunohistochemistry	Yes	[62]
		RT-PCR	Yes	[129]
		RT-PCR	None	[122]
	Rat hepatic artery	RT-PCR	Yes	[130]
	Rat mesenteric vein	Immunohistochemistry	Yes	[54, 60]
	Guinea-pig arterioles of the ileum submucous plexus	Immunohistochemistry	Yes	[184]
	Rat and mouse pancreatic vascular bed	Immunohistochemistry	Yes	[29]
<b>P2X<sub>2</sub></b>	Rat mesenteric artery and vein	Immunohistochemistry	Low density	[60]
			Strongly stained	[29]
	Rat and mouse pancreatic vascular bed	RT-PCR	None	[122]
	Rat mesenteric artery		Weak	[129]
	Rat hepatic artery		Weak	[130]
<b>P2X<sub>3</sub></b>	Rat hepatic artery	RT-PCR	Weakly expressed	[130]
	Rat mesenteric artery		Weakly expressed	[129]
<b>P2X<sub>4</sub></b>	Rat hepatic artery	RT-PCR	Strongly expressed	[130]
	Rat mesenteric artery		Very strongly expressed	[129]
<b>P2X<sub>5</sub></b>	Rat hepatic artery	RT-PCR	Not expressed	[122]
			Strongly expressed	[130]
<b>P2X<sub>6</sub></b>	Rat mesenteric artery	RT-PCR	Strongly expressed	[129]
			Weakly expressed	[129]
<b>P2X<sub>7</sub></b>	Rat hepatic and mesenteric arteries	RT-PCR	Never detected	[129, 130]
	Rat hepatic artery	RT-PCR	Strongly expressed	[130]
<b>P2Y<sub>12</sub></b>	Rat mesenteric artery	RT-PCR	Strongly expressed	[129]
	Rat mesenteric artery	RT-PCR	Yes	[21]
	Mouse mesenteric artery	RT-PCR	Yes	[177]
	Rat and mouse pancreatic vascular bed	Immunohistochemistry	Yes	[29]
	Rat mesenteric vein	Functional study	Yes	[54]
<b>P2Y<sub>4</sub></b>	Guinea pig mesenteric vein	RT-PCR	Yes	[116]
			None	[21]
<b>P2Y<sub>6</sub></b>	Rat mesenteric artery	RT-PCR	Yes	[21]
	Mouse mesenteric artery	RT-PCR	Yes	[177]

hypothesis of different storage has also been put into discussion in the rat mesenteric artery [41]. Differences in the methods used to quantify noradrenaline and ATP may account for the ambiguous results since the electrochemically measured noradrenaline may originate from both “small” and “large” dense cored vesicles, whereas excitatory junction potentials are believed to result only from ATP released from “small” dense cored vesicles [162].

It is generally accepted that periarterial nerve stimulation at low frequencies mostly causes a release of purinergic transmitter whereas stimulation at high frequencies mainly induces a release of adrenergic transmitter [142–144, 187, 192]. Modulation of ATP release by noradrenaline can only be revealed during nerve stimulation of very low frequencies ( $0.2 \pm 2$  Hz) and short duration (10 s) [9, 117]. Parallel with what has been described on transmitter release, the neurogenic contractions induced by electrical field stimulation of postganglionic sympathetic nerves within splanchnic vessels are biphasic [16, 94, 144, 154]. In dog [115] and

rabbit mesenteric arteries [181], the first component is purinergic in nature, develops rapidly, is transient and predominates at low frequencies (1–2 Hz) of stimulation, whereas the second component is adrenergic, develops after the purinergic one, is more long-lasting and becomes more evident as the frequency of stimulation increases (10 Hz). Based on these findings, it was speculated that the purinergic component is involved more dominantly in the early phase of the total response, while the adrenergic component mainly contributes to the latter phase [115]. The fact that exogenous ATP produces a rapid and transient contraction and mimicked the neurogenic purinergic response suggested that the profile of neurogenic purinergic transmission reflects postjunctional rather than prejunctional mechanisms [115]. However, it has also been suggested that ATP released from the endothelium may function as a modulator of sympathetic neurotransmission and contribute to the purinergic constriction component, since the removal of the endothelium decreased the



purinergic component but not the adrenergic component of the vasoconstrictor responses to stimulation [191]. Functionally, neurogenic released ATP evokes biphasic responses on the vascular smooth muscle: initially direct excitatory junction potentials followed by indirect hyperpolarization through the release of endothelium-derived hyperpolarizing factor (EDHF) [172]. Interestingly, there is a report of ATP being partially responsible for the neurogenic NANC endothelium-independent relaxation of the longitudinal muscle of the rabbit portal vein [13]. This ATP-mediated neurogenic relaxation seems to come about via P2 receptors located on the smooth muscle [13, 76]. A study suggested that the sympathetic purinergic contractile responses of the dog mesenteric artery may be caused by an unknown substance selective to P2X-purinergic receptors, rather than ATP [114]. Alternatively, neurogenically released ATP may act only on the P2X-purinergic receptors, unlike exogenously ATP [114].

An important point concerning differential proportions of the purinergic and adrenergic components of the sympathetic neurotransmission refers to the calibre of the vessels in question. This issue is effectively evaluated in the mesenteric vasculature where it is possible to study vessels with quite different internal diameters. It is generally agreed that the importance of the purinergic component increases and that of the adrenergic component decreases with the reduction of the size of the mesenteric arteries [56, 93]. If one thinks that in small mesenteric arteries the neurogenic purinergic component of the periarterial nerve stimulation dominates [56, 93], that small mesenteric arteries are more sensible to  $\alpha, \beta$ -meATP and UTP than large arteries [57], and even more, that in small (but not large) mesenteric arteries both P2X<sub>1</sub> and P2Y<sub>2</sub> receptors are found (see references below), it makes sense that neurally released ATP in this small vessels induces an immediately direct vasoconstriction, through activation of P2X<sub>1</sub> receptors and a later indirect endothelium-dependent vasorelaxation via activation of P2Y<sub>2</sub> receptors and the release of EDHF [172].

Sympathetic cotransmission also seems to be dependent on the species and/or regional tissue studied. As previously pointed out, the majority of the studies show that low frequencies of stimulation favour purinergic neurotransmission while high frequencies promote adrenergic neurotransmission [142, 143, 187, 192]. A variation was found in the canine gastropiploic [170] and splenic artery [192]. In these preparations, the first phase is mediated by P2X receptors at low frequencies of stimulation, but also presynaptic  $\alpha$ -adrenoceptors at high frequencies, while the second phase response involves mostly  $\alpha_1$ -adrenoceptors and partially P2X receptor activations at all frequencies used. Uniquely, electrical stimulation (100 pulses, 10 Hz) of the guinea-pig submucosal arterioles caused vasoconstriction mediated by activation of P2X receptors by ATP or a

related purine nucleotide [47]. The functional role of neurally released noradrenaline in this tissue appears to be restricted to its prejunctional modulation of transmitter release [47].

The electrical and contractile response elicited with stimulation of sympathetic nerves has been shown to differ significantly between arteries and veins. For instance, in the guinea-pig mesenteric artery, sympathetic nerve stimulation gives rise to rapidly rising excitatory junction potentials of brief duration that are resistant to prazosin, but are blocked by  $\alpha, \beta$ -meATP [67, 82]. In contrast, in the guinea-pig mesenteric vein a slow depolarization [166] that is resistant to purinoceptor desensitization with  $\alpha, \beta$ -meATP [65, 67] is observed rather than fast EJPs. This has led to the conclusion that ATP is not involved in these junctional events [65, 67] and, hence, is not released during nerve stimulation. However, a study on guinea-pig mesenteric vessels [155] revealed that arterial contractile response to sympathetic nerve stimulation is due exclusively to noradrenaline, whereas at least three different neurotransmitters (noradrenaline, ATP and neuropeptide Y) appear to contribute to the venous neural response. In another study, electric field stimulation induced ATP overflow in the guinea-pig mesenteric vein [8]. The difference in the contribution of ATP to the neural response in artery and vein was suggested to be related to the presence of different populations of ATP receptors, i.e., in artery, rapidly desensitizing P2X<sub>1</sub> receptors predominate whereas in vein, non-desensitizing P2Y receptors predominate [116]. However, the apparent contradictory results between these different studies [67, 82, 155] may also be ascribed to differences in the methodological approaches (evaluation of membrane potentials by electrophysiology *vs* contractile and [<sup>3</sup>H]-noradrenaline release studies) or to the size of the vessels studied (the studies of Kreulen [82] involved a broader range of internal diameters of the vessels). Besides, there might be differences in the postjunctional effects of prejunctionally released neurotransmitters, as it is suggested to occur in the human saphenous vein [147], where ATP is co-released with noradrenaline but did not appear to generate the non-adrenergic component of contraction. In the guinea-pig, release of both ATP and noradrenaline in mesenteric veins exceeds release in arteries [8]. Differently, in the dog, release of noradrenaline in veins exceeds release of noradrenaline in arteries, whereas both vessels release equal amount of ATP [9].

Whether ATP by itself or indirectly after degradation to adenosine modulates sympathetic neurotransmission is another point of interest in the context of this review. Inhibition of sympathetic neurotransmission via prejunctional P2 receptors has been described in a number of tissues from rat and mouse [182] although little investigation has pursued this issue in the splanchnic vasculature. In

the dog mesenteric artery  $\alpha\beta$ -meATP had no effect on [ $^3$ H] noradrenaline release, suggesting that the P2 purinoceptor is absent or not functioning in the sympathetic nerve terminals [115]. In the rat mesenteric artery, ATP inhibition of noradrenaline release occurs without the conversion of ATP to adenosine [153]. However, the inhibition was completely abolished by DPCPX, an antagonist of adenosine A<sub>1</sub> receptors, and pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS), an antagonist of P2 receptors, suggesting that the receptor involved may be different from the well known adenosine A<sub>1</sub> or P2-receptors [153]. On the contrary, in the hamster mesenteric arterial bed, prejunctional adenosine A<sub>1</sub>, but not P2, receptors modulate sympathetic neurotransmission [136]. The purinergic component of sympathetic neurotransmission does not contribute significantly to sympathetic contractile responses of this vascular bed [136, 139]. It was suggested that inhibitory P2 receptors are expressed selectively on sympathetic nerves utilizing ATP as a cotransmitter, in accordance with the view that prejunctional P2 receptors on sympathetic nerve terminals are release-modulating autoreceptors [178, 183]. Another possibility is that endothelium-derived factors released upon activation of endothelial UTP-sensitive P2Y receptors inhibit ATP release from purinergic nerves in the smooth muscle cells of hamster mesenteric artery [171].

ATP elicits arterial contraction through stimulation of P2X receptors in the mesenteric artery of the rat [54, 73, 85, 123, 137], guinea-pig [116] and rabbit [20], and in the rat hepatic artery [130] and the guinea-pig portal vein [125]. The P2X<sub>1</sub> subtype is the receptor responsible for the purine nucleotides-induced arterial contractions [54, 177]. In contrast to what is observed in the arteries, venoconstriction is mainly mediated by P2Y<sub>2</sub> receptors [54, 116, 123], although P2Y<sub>1</sub> receptors (and possibly P2Y<sub>4</sub> and P2Y<sub>6</sub> receptors) and to smaller extent P2X<sub>1</sub> receptors [116] may also play a role. In the perfused rat mesenteric artery, a residual contractile response to ATP was observed after exposure to P2X antagonists [123]. Also, in isolated 2nd-order rat mesenteric arteries UTP-induced contractions persisted after desensitization of P2X<sub>1</sub> receptors [54]. These studies suggest that other P2 receptor(s), probably P2Y<sub>2</sub> receptors, contribute to the arterial contractile effect of purine nucleotides although playing a minor role [54, 123]. A P2Y receptor-mediated contractile effect has been described previously although not in the splanchnic circulation [63, 149, 179]. More recently, a study in mesenteric arteries from P2X<sub>1</sub>-KO mice revealed that although P2X<sub>1</sub> receptors underlie an important component of the sympathetic neurogenic contraction, UTP and ADP, but not ATP, evoked concentration-dependent arterial contraction [177], suggesting the presence of a UTP- and UDP-sensitive P2Y receptor. These authors propose the P2Y<sub>6</sub> receptor as a candidate for mediating this sustained vasoconstriction

[177]. However, a very recent work of the same group [86] concluded that P2X<sub>1</sub> receptors are required to generate the junctional calcium transients that activate this component of the neurogenic contraction, since they were not observed in P2X<sub>1</sub>-KO mice. This observation doesn't exclude the possibility raised in previous studies of P2X<sub>1</sub> receptors being the principal mediators of purinergic contraction with other receptors playing minimal roles. In line with the view that both P2X and P2Y receptors participate in vasoconstriction is the study of Lagaud and co-workers [85]. These authors described that both P2X- and P2Y-receptor mediated arterial contractions through different mechanisms. P2X receptors are activated by low concentrations of ATP ( $\mu$ M range) and induce depolarization of the cell leading to the opening of voltage-dependent Ca<sup>2+</sup> channels and direct calcium influx. Additionally, P2Y-purinoceptors are activated by high concentrations of ATP (mM range) which promote first a calcium influx through activation of a PTX-sensitive G protein and a dihydropyridine-sensitive mechanism, and secondly a release of calcium from intracellular stores via the phospholipase C/IP3 pathway. In contrast, a study in the guinea-pig small mesenteric arteries couldn't observe P2X-mediated effects [108]. The dissimilar results obtained concerning the exclusivity of P2X<sub>1</sub> receptors or the result of both P2X/P2Y receptors in mediating purinergic contraction probably reflect differences between species, as previously suggested [177].

The seven P2X subunits can form functional homomeric or heteromeric P2X receptors (see Introduction). The finding of mRNA for some P2X receptors with no evidence for a functional role [130] and the pharmacology evidence for some yet unidentified P2X receptors [57, 175] has been suggesting that arteries may express heteromeric P2X receptors with properties dominated by the P2X<sub>1</sub> receptor subunit. The blood pressure of P2X<sub>1</sub> receptor-deficient mice was normal or slightly elevated [112], suggesting that P2X<sub>1</sub> receptors may not be essential for the expression of arterial P2X receptors, although P2X receptors are not the only receptors that regulate blood pressure levels. On the contrary, in P2X<sub>1</sub>-KO mice ATP-mediated vasoconstrictions were abolished [177], suggesting that P2X<sub>1</sub> receptor subunit is essential for the production of functional P2X receptors. However, one has to consider the possibility that there is compensation of other P2X receptors in these animals.

Activation of endothelial P2Y receptors causes arterial dilation [137]. This vasodilation is probably mediated by P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors, through hyperpolarization of the endothelium that correlates with endothelium-dependent vasodilation [105]. This P2Y receptor-induced endothelium-mediated vasodilation occurs through the release of NO, via the NO/cGMP pathway [21], and cyclo-oxygenase metabolites [97, 105] but also via the release of EDHF [97, 98, 99,

[105, 148]. In fact, EDHF-mediated vasodilation seems to be the predominant mediator of P2 receptor-mediated regulation of perfusion pressure in the small arteries of the peripheral circulation [96, 172]. Both NO- and EDHF-mediated vasodilation can be induced by activation of P2Y<sub>1</sub>, P2Y<sub>2</sub> and P2Y<sub>4</sub>, but not P2Y<sub>6</sub>, receptors [95–98].

Some important features are associated with the endothelial P2Y-induced EDHF-mediated vasodilation. First, it is not observed in all vessels. Malmström and co-workers [97] reported that it occurs in the rat mesenteric artery but not in the carotid artery. Second, it depends on the resting tone of the vessel. In rat mesenteric arteries it was only observed in arteries which were under a low resting tone (1 mN) [97]. Finally it may vary dramatically according to the vessel's internal diameter. P2Y<sub>2</sub> receptor-induced EDHF-mediated relaxation was clearly evident in large diameter main branch of the rat mesenteric artery (internal diameter of about 1.24 mm) but almost absent in the rat small mesenteric arteries (internal diameter of about 340 µm) [105]. In another study [172], electrical stimulation or adventitial application of ATP induced endothelium-dependent hyperpolarization in thin hamster mesenteric arteries (internal diameter < 150 µm) but not in thick arteries (internal diameter > 300 µm). Usually, the importance of EDHF versus nitric oxide generally increases as the vessel diameter is reduced [152]. The results of Thapaliya and co-workers are more coherent with this view. However, as the stimulus was electrical stimulation or adventitial application of ATP, the higher number of smooth muscle cell layers was probably determinant for the absence of hyperpolarization in thick mesenteric arteries. This hypothesis is reinforced by the fact that intimal application of ATP could induce endothelium hyperpolarization [172]. In what concerns the study of Mistry and co-workers, differences in the distribution and/or transduction signalling of the endothelial P2Y<sub>2</sub> receptors, a counteracting effect of constrictor P2Y<sub>2</sub> receptors in the smooth muscle cell or other P2 receptors, namely P2X receptors, could mask the vasodilatory effect in small mesenteric arteries. Indeed, activation of P2X receptors counteracts the hyperpolarization of vascular smooth muscle cells mediated by P2Y-induced release of EDHF [98, 99] but not NO [96]. Furthermore, species differences may also account for the different profile of the two studies. Curiously, in arteries of about 350 µm, neither electrical stimulation or adventitial administration of ATP induced hyperpolarization [172] nor stimulation of P2Y<sub>2</sub> receptors with ATPγS caused vasodilation [105].

At a glance, it seems that the effects of ATP on the vasculature are quite clear-cut. P2X receptors, namely P2X<sub>1</sub> present on vascular smooth muscle cells, are responsible for the ATP-induced vasoconstriction, while P2Y receptors, mainly P2Y<sub>2</sub> located on vascular endothelial cells, mediate ATP-induced vasodilation. However, the effects of ATP on

vessels are much more multifaceted. Recent reports of ATP inducing endothelium-independent relaxations [59, 135] and the evidence for the presence of P2X<sub>1</sub> receptors on vascular endothelial cells [62] increased the complexity of the effects of ATP on splanchnic circulation. ATP can induce three types of response in mesenteric arterial bed of the rat [62, 72, 135, 161]: (1) contraction, mainly mediated by smooth muscle P2X<sub>1</sub> receptors, (2) rapid and transient relaxation, mainly mediated by P2Y<sub>2</sub> receptors, and (3) slow and sustained relaxation. The mechanism responsible for this prolonged relaxation is controversial and has only very recently started to be characterized [62, 134, 135]. Guibert and co-workers [59] described an endothelium-independent ATP-induced relaxation that was attributed to the conversion of ATP to adenosine by ectonucleotidases [59]. The first study that tried to characterize the receptor mediating this sustained possibly endothelium-independent relaxation induced by ATP was signed by Ralevic [135]. This study couldn't conclude on the purinergic receptor involved. In a second study, Ralevic [134] concluded that smooth muscle P2X receptors were responsible for a biphasic response: contraction and prolonged relaxation, although a role of P2Y receptors could not be excluded, since the sustained relaxation was not abolished by P2X receptor desensitization [135]. Concurrently with the studies of Ralevic, prolonged relaxation to ATP has been reported to be abolished by removal of the endothelium [161]. In a even more recent study [62], it was clearly shown that α,β-mATP induced endothelium-dependent vasodilation which was also independent from the contractile effect. The preparation and methods used to remove the endothelium can explain, at least in part, the opposite results. While Ralevic used the isolated perfused mesenteric vascular bed, a preparation in which it is difficult to remove the endothelium, and endothelium removal with distilled water, Stanford and co-workers used sodium deoxycholate to endothelium-denude the arteries. In the study of Harrington and Mitchell, however, isolated rings of 2nd-order rat mesenteric arteries (mean internal diameter of 247 µm) were mounted on a wire myograph and the endothelium was physically removed by a human hair and a permanent control of the process was performed to ensure that it didn't lesion the smooth muscle cells. Moreover, it should be noticed that the study of Guibert was the only in which a vein (the rat portal vein) was used. As happens with other situations, there can be differences in the distribution and function of purinergic receptors between the arterial and the venous side of the circulation. The study of Harrington and Mitchell further evidenced, for the first time, the presence of P2X<sub>1</sub>-like immunoreactivity in the surface of the endothelium, and concluded that the ATP-induced endothelium-dependent dilation was mainly mediated by P2X<sub>1</sub> receptors through the release of EDHF. In this context, it is interesting to

**Table 4** Potencies of agonists and antagonists at P2X and P2Y receptors in the splanchnic circulation

Agonist/Antagonist	Slope	pEC <sub>50</sub>	EC <sub>50</sub> (μM)	pEC <sub>25</sub>	pEC <sub>25'</sub>	pA <sub>2</sub>	Reference
<i>P2X receptors</i>							
α,β-meATP	52.9(±12.8)			8.93			[20]
		6.68±0.13					[139]
			0.7±0.4				[72]
							[85]
β,γ-meATP	44.3(±11.8)						[20]
2MeSATP	28.6(±10.8)						[20]
		5.71±0.22		7.09			[139]
			60±25				[72]
							[85]
ATP	31.3(±5.4)						[20]
		4.94±0.15		6.13±0.10			[139]
			280±105				[72]
ADP				6.00±0.16			[85]
			254±50				[139]
UTP		4.14±0.09		5.76±0.17			[139]
			79±6				[72]
							[85]
Suramin						4.6; 4.89	[139]
<i>P2X<sub>1</sub> receptors</i>							
α,β-meATP			0.3±0.1				[54]
	1.6±0.4; 4.2±1.5		0.4; 2.5				[57]
	1.3±0.1	5.8±0.1					[90]
			0.28±0.04				[116]
ATP			196±64				[54]
			310±30				[116]
UTP			24±5				[54]
			8±0.4				[116]
2MeSATP			30±2.7				[116]
ATPγS			29±3				[116]
ADP			1900±140				[116]
βγDMeATP			20±8				[116]
βγLMeATP			31±9				[116]
UDP			250±3				[116]
<i>P2Y receptors</i>							
β,γ-meATP	33.0(±5.5)						[20]
2MeSATP	40.6(±8.8)						[20]
			33±7				[85]
			1.22±0.18				[137]
ATP	44.4(±9.3)						[20]
			933±100				[85]
			11.8±0.18				[137]
ADP			1403±324				[85]
<i>P2Y<sub>1</sub> receptors</i>							
MRS2179	0.71±0.09					7.1	[105]
ADPβS		6.56±0.1; 6.29±0.31					[105]
<i>P2Y<sub>2</sub> receptors</i>							
ATP			2.8±0.9				[54]
UTP			15±4				[54]
	1.1±0.3; 2.2±0.3		15; 88				[57]
ATPγS		6.92±0.21					[105]
<i>P2Y<sub>1</sub>/P2Y<sub>2</sub> receptors</i>							
α,β-meATP		7.5±0.1					[21]
2MeSATP		8.0±0.2					[21]
2MeSADP		8.2±0.2					[21]

**Table 4** (continued)

Agonist/Antagonist	Slope	pEC <sub>50</sub>	EC <sub>50</sub> (μM)	pEC <sub>25</sub>	pEC <sub>25</sub> '	pA <sub>2</sub>	Reference
ATP		6.1±0.1					[21]
ADP		6.8±0.1					
UTP		5.9±0.1					[21]
UDP		6.0±0.1					[21]
<i>P2Y<sub>1</sub>/P2Y<sub>2</sub>/P2Y<sub>4</sub> receptors</i>							
UTPγS		6.2±0.1					[95]
ADPβS		6.0±0.1					[95]
		6.6±0.1					[96]
		6.5±0.1					[98]
ATPγS		5.8±0.2					[95]
UTP		4.8±0.2					[95]
		6.8±0.4					[96]
		6.1±0.2					[98]
ADP		5.3±0.2					[95]
ATP		5.4±0.2					[95]
		6.4±0.2					[96]
		5.9±0.2					[98]
2MeSATP		5.9±0.1					[98]
UDP		3.8±0.3					[95]
		4.2±0.1					[98]
<i>P2Y<sub>6</sub> receptors</i>							
α,β-meATP	2.7	6.1±0.2					[95]
		6.76±0.09					[177]
ATP	2.4	4.1					[95]
		4.75±0.12					[177]
UTPγS		5.3±0.2					[95]
UTP		4.74±0.15					[177]
ATPγS		4.1±0.2					[95]
UDPβS					7.2±0.2		[95]
UDP					2.8±0.2		[95]
		4.97±0.11					[177]

Predominant receptor considered to be involved on the basis of functional data and receptor localization studies. Values ± S.E.M. or 95% confidence interval (in parenthesis). Slope refers to the slope of the concentration-response curve; EC<sub>50</sub> represents the molar concentration of the agonist needed to cause 50% of the maximum response; pEC<sub>50</sub> and pEC<sub>25</sub> are the negative logarithms to base 10 of the EC<sub>50</sub> and EC<sub>25</sub> of an agonist, respectively; pEC<sub>25</sub>' is the negative logarithm to base 10 of the concentration of the agonist needed to cause an increase of 25 mmHg in blood pressure; pA<sub>2</sub> is the negative logarithm to base 10 of the molar concentration of the antagonist that makes it necessary to double the concentration of the agonist needed to elicit the original response.

β,γ-methylene ATP (β,γ-meATP); 2-methylthio ATP (2MeSATP); adenosine 5'-O-3-thiotriphosphate (ATPγS); N6-methyl-2'-deoxyadenosine-3',5'-bisdiphosphate (MRS2179); adenosine 5'-O-thiodiphosphate (ADPβS); adenosine 5'-O-3-thiotriphosphate (ATPγS); 2-methylthioADP (2-MeSADP); uridine 5'-O-3-thiotriphosphate (UTPγS); uridine 5'-O-thiodiphosphate (UDPβS); α,β-methylene ATP (α,β-meATP); adenosine triphosphate (ATP); adenosine diphosphate (ADP); uridine triphosphate (UTP); uridine diphosphate (UDP).

notice that renal arteries, which express P2X<sub>1</sub> receptors on the smooth muscle cells but not on endothelial cells, contract without subsequent vasodilation in response to α,β-meATP [80].

Table 4 summarizes the information on the potencies of agonists and antagonists at P2X and P2Y receptors in the splanchnic circulation.

Mistry and co-workers suggested the existence of interactions between P2Y receptor subtypes. They observed that in rat mesenteric artery, relaxation of smooth muscle cells and hyperpolarization of endothelial cells caused by

the P2Y<sub>2</sub> receptor agonist ATPγS were potentiated in the presence of MRS 2179, an antagonist of P2Y<sub>1</sub> receptors [105], as previously reported in non-splanchnic vessels [27, 35, 128].

### Future perspectives

This review summarized the data on the distribution and function of purinergic receptors particularly in vessels of the splanchnic circulation. The splanchnic circulation receives



about one fourth of the cardiac output. Blood flow through the superior mesenteric artery significantly increases in a post-prandial situation [168]. In humans, the gastrointestinal tract retains its high blood flow priority, even in conditions of dynamic muscular exercise or in a pressor situation [45, 185]. Thus, the dysregulation of splanchnic vascular tone is likely to contribute not only to splanchnic organs dysfunction, but also to the development of marked hemodynamic effects (e.g. altered mean arterial pressure, heart rate and total peripheral resistance) and organ failure. In intensive care patients, inadequate splanchnic perfusion is associated with increased morbidity and mortality [81].

Purinergic receptors actively participate in the regulation of splanchnic blood flow and may play an important role in clinically relevant pathological states. For instance, adenosine A<sub>1</sub> receptor activation seems to contribute to the improvement of splanchnic perfusion in adjunctive direct peritoneal resuscitation [193]. Purinergic receptors play an important role in the regulation of vascular and immune cell-mediated responses. These receptors trigger and mediate acute processes that affect cellular metabolism, adhesion, activation or migration. Repetitive stimulation of purinergic receptors also causes long-term reactions, like cell proliferation, differentiation and apoptosis, as seen in several chronic inflammatory states. In the cardiovascular system, purines can influence blood pressure, platelet activation, thrombosis, inflammatory processes, cardiac function and vasomotor responses [10, 11, 40, 50]. The study of purinergic receptors in the splanchnic circulation is a growing field of interest. More research is needed in order to, in a near future, originate new drugs specifically designed to treat pathologies like portal hypertension, ischemic bowel syndrome and several inflammatory states.

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